

FIG. 1A

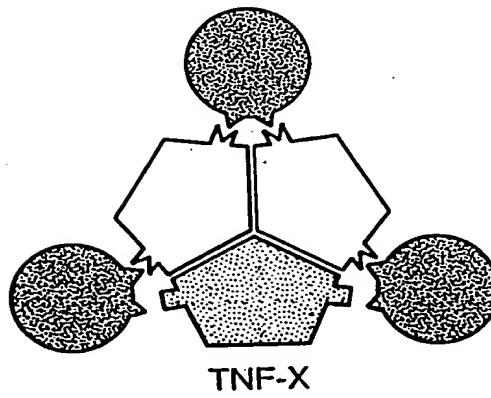


FIG. 1B

09981289 101501

FOSTOT" 682TB660

TNF-TNFR TRIMER COMPLEX

TOP VIEW



SIDE VIEW

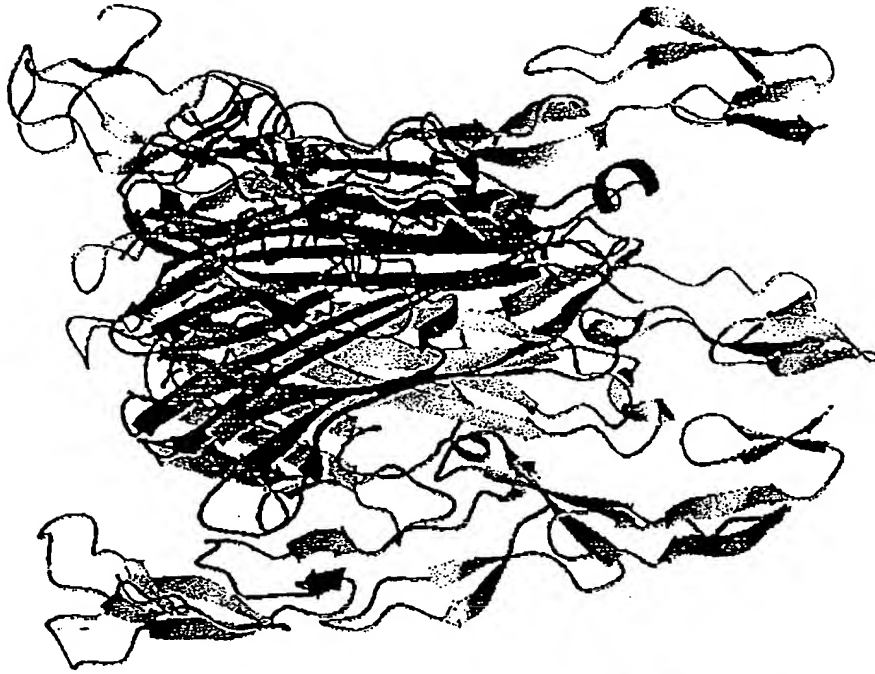


FIG.-2

FOOT " 6827860

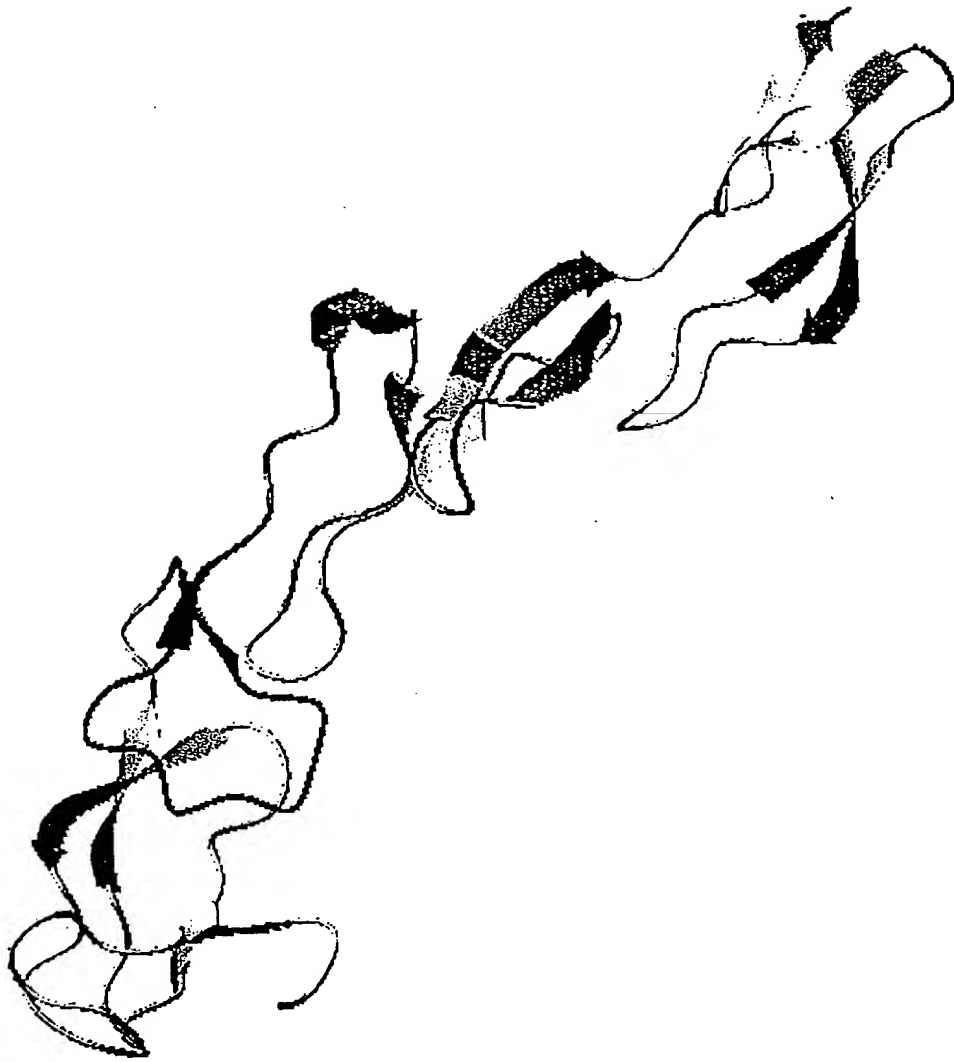
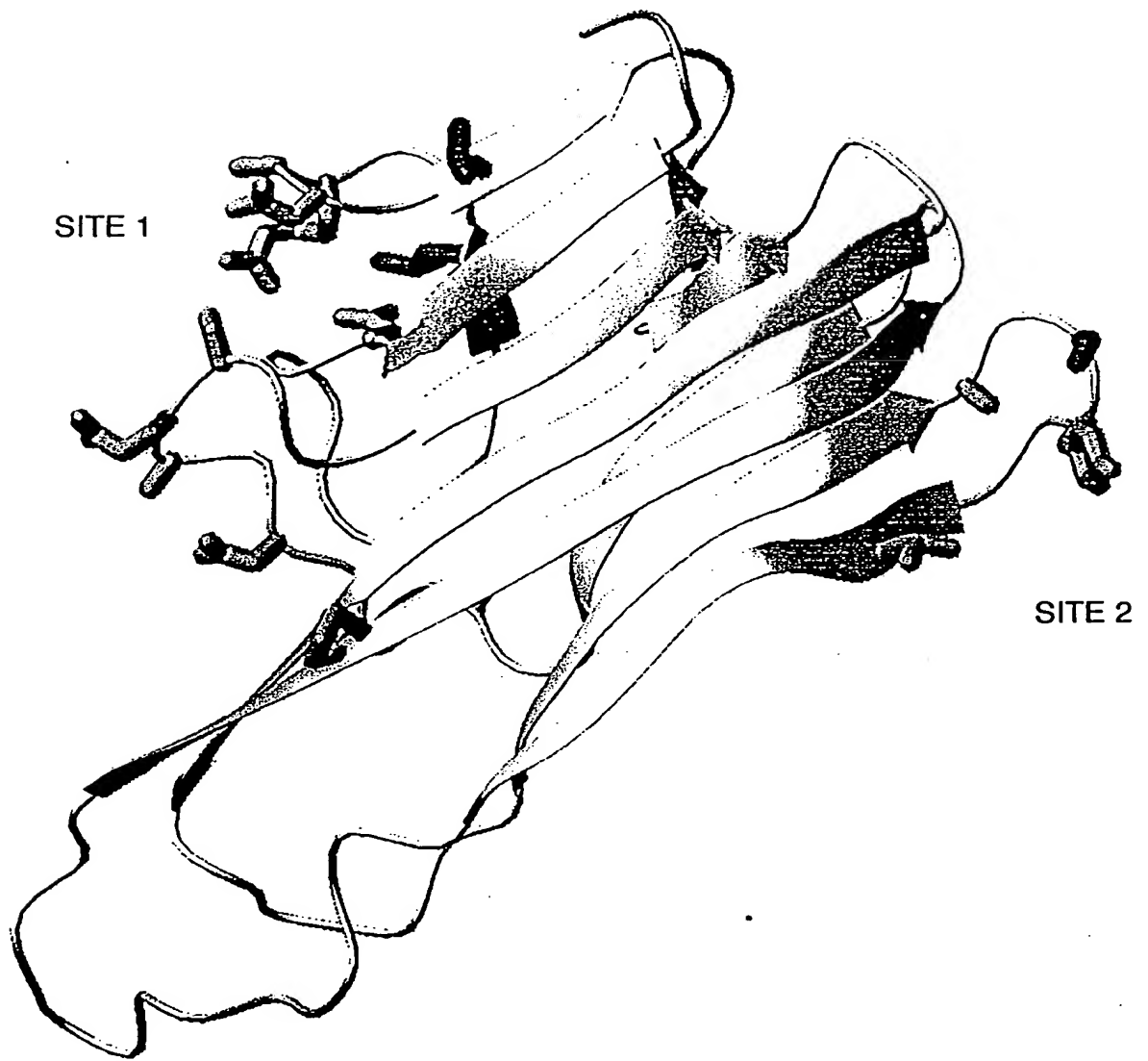
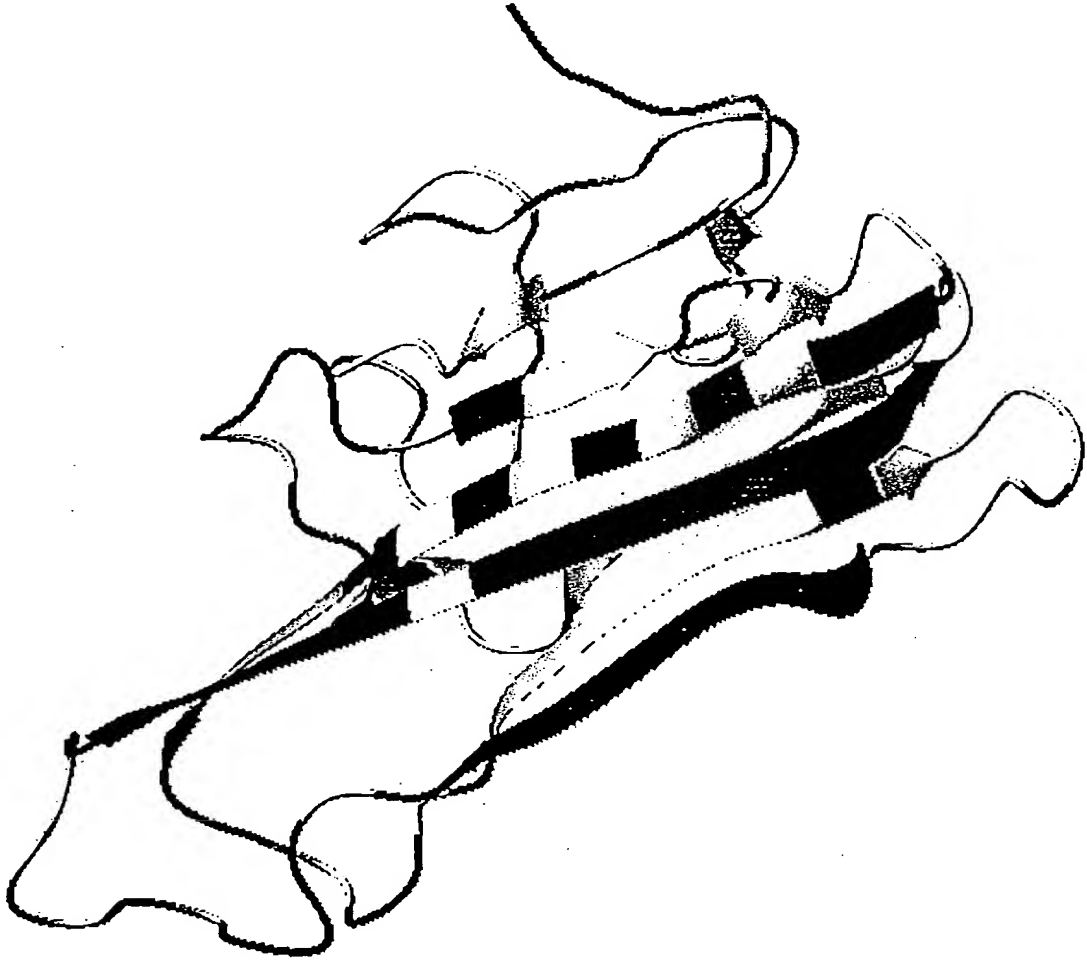


FIG. 3

TNF α BINDING SITES**FIG._4**

TOSTOT 682T8660

TNF α TRIMER INTERFACE**FIG._5**

FOSTOT" 682T8660

1 atgcaccacc ac cacca cgtacgctcc tctcccgcga ggtccga caaaccggta
 61 gctcacgtag tagetaaccc gcaggctgaa ggtagctgc ggtgaa cgcgcgcgct
 121 aacgctctgc tggctaaccg tgtagaactg cgcgacaacc agctggtagt accgtccgaa
 181 ggtctgtacc tgatctactc ccaggctactg ttcaaaggctc aggggtgtcc gtccactcac
 241 gtactgctga ctacactat ctcccgcacg gctgtatcct accagactaa agtaaaccctg
 301 ctgtccgcta tcaaattccc gtgtcagcgc gaaactccgg aagggtgctga agctaaaccg
 361 tggtagaac cgatctacct ggggtggtgta ttccagctgg aaaaagggtga cgcctgtcc
 421 gctgaaatca accgcccggg ctacctggac ttcgctgaat ccggtcaggt atacttcggt
 481 atcatcgctc tgtga

FIG._6A

1 MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRR NALLANGVEL RDNQLVVPSE
 61 GLYLIYSQVL FKQGQCPSTH VLLTHTISRI 'AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP
 121 WYEPIYLGGV FQLEKGDRLS AEINRPDYLD FAESQVYFG IIAL

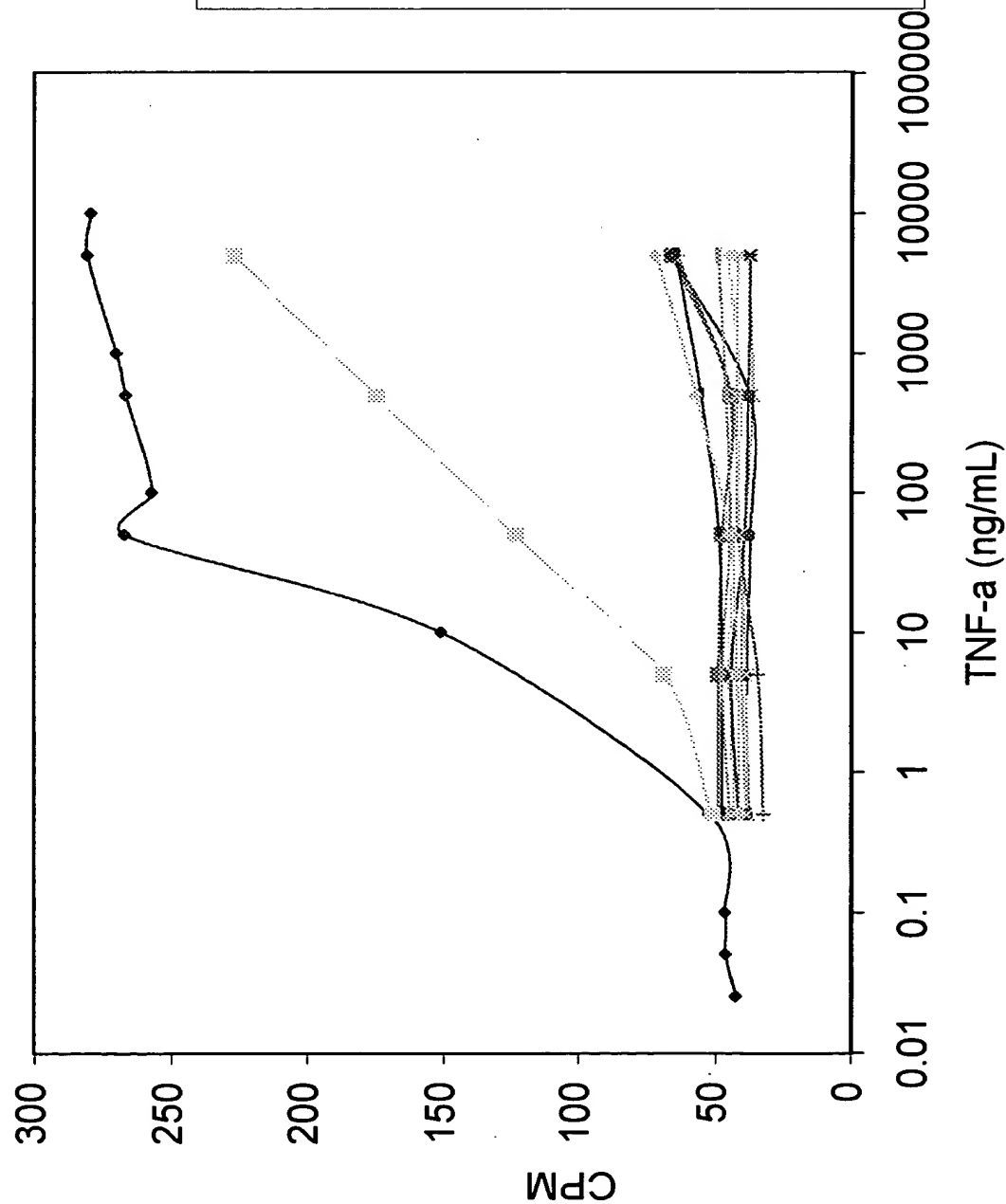
FIG._6B

Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants created
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

ALSO MADE DOUBLE MUTANTS K65E/D143K, K65E/D143R, K65D/D143K AND K65D/D143R

FIG._7

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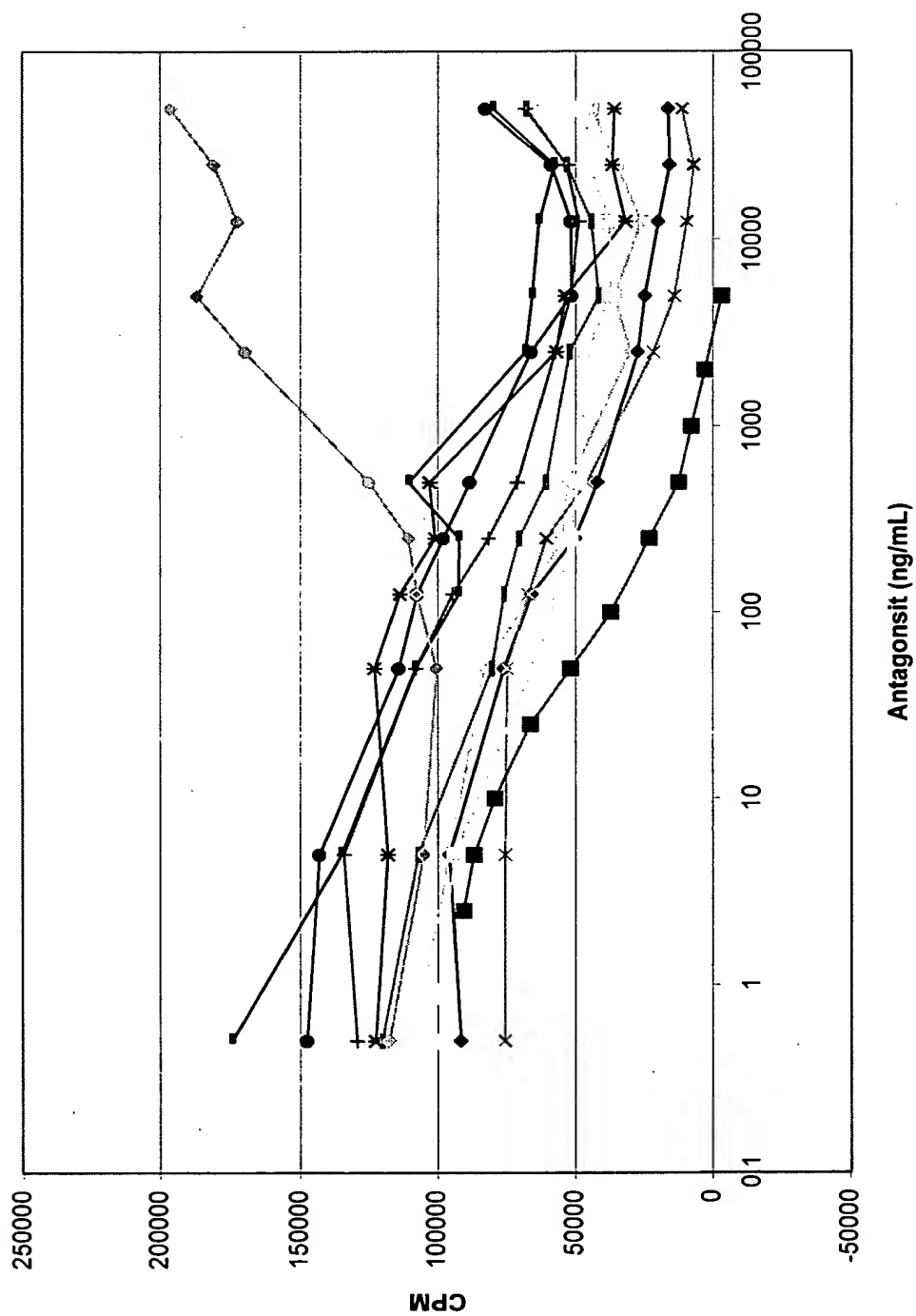


Fig 10a

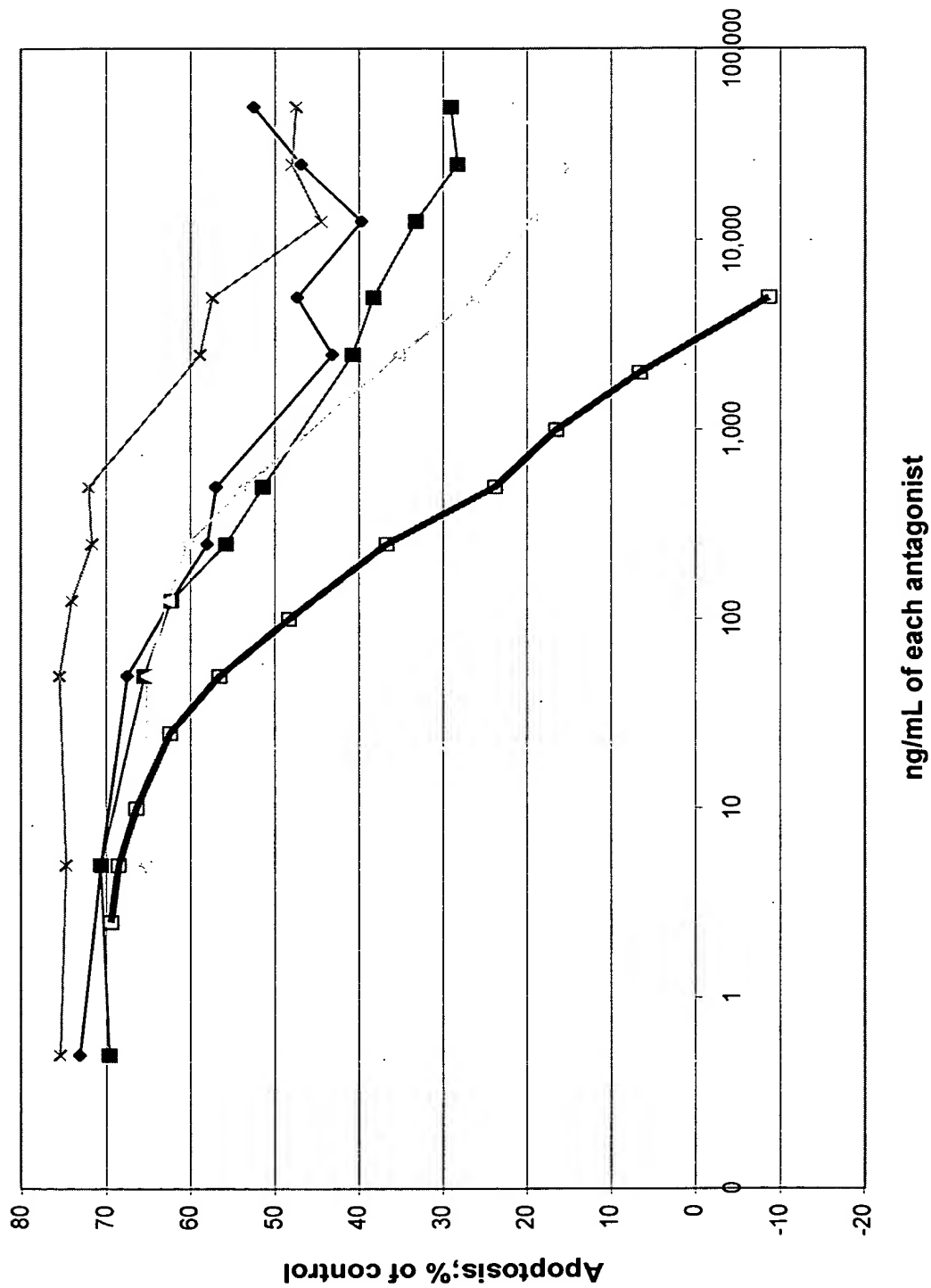
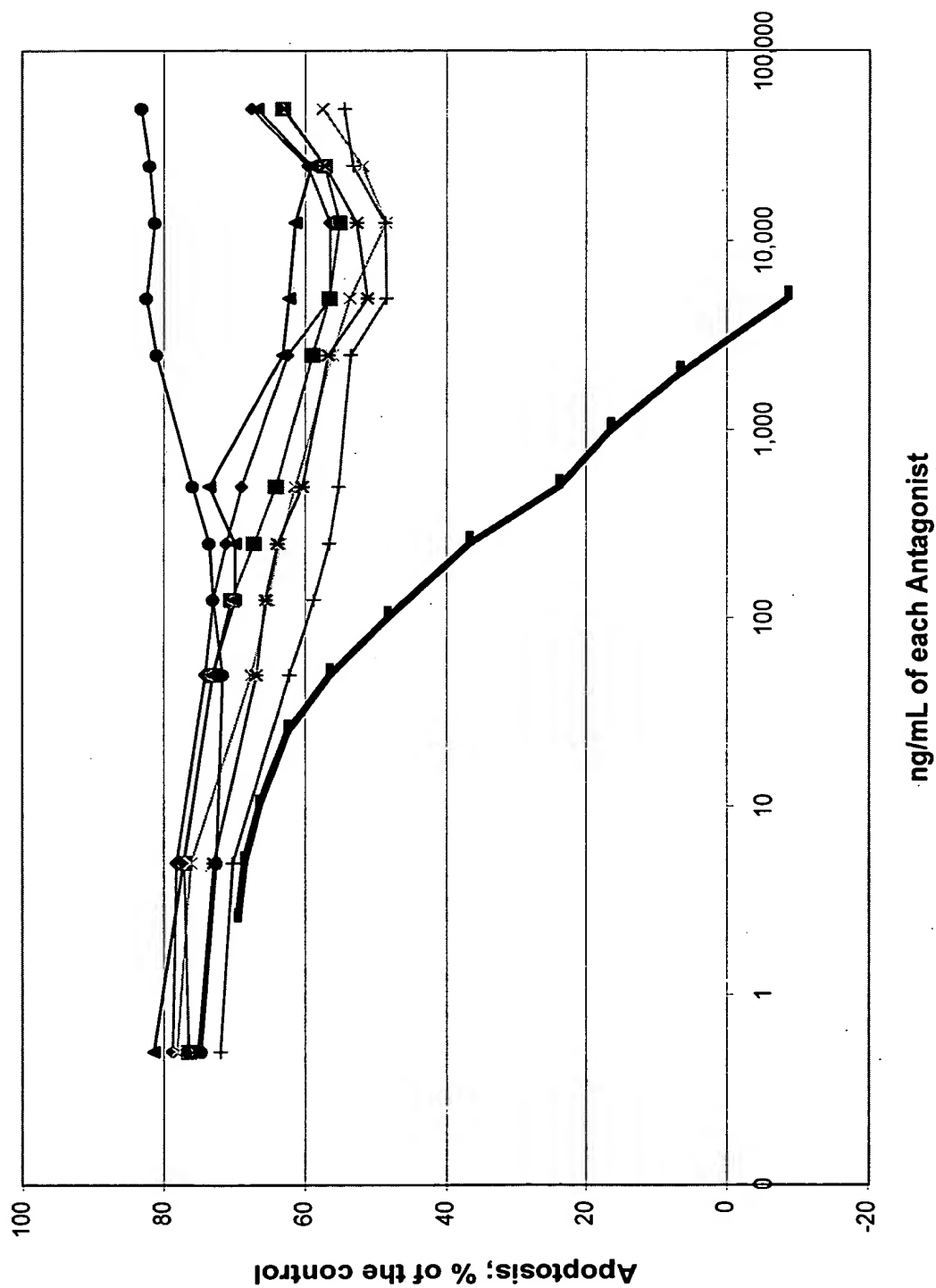


Fig-10b



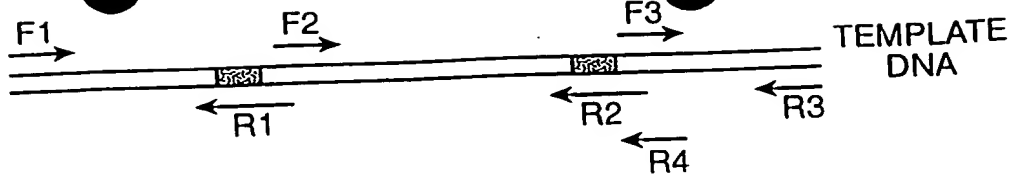
POST "GETB60"

WT	PDA Relative Probability Distribution																
Q21	R1000																
N30	D1000																
R31	I1000																
R32	H1000																
A33	E1000																
A35	S1000																
K65	R585	D146	K110	T42	H31	M27	W15	I15	Q10	S9	N9	V1					
G66	Q813	K187															
Q67	D623	W209	Y83	R43	K41	S1											
A111	R959	E41															
K112	K1000																
Y115	Q230	K154	E116	N84	Y81	R72	F69	H43	M39	L36	I26	W25	D11	T8	S6		
D140	D1000																
L143	D680	E130	N110	Q33	S29	R12	K6										
F144	F695	N305															
A145	R456	D196	K124	N76	H67	T43	Q25	E9	Y1	M1	S1	F1					
E146	N489	K377	R111	D12	S10	E1											
S147	R1000																

FIG. 11

+

BLACK BOX =
REGION TO
BE MUTATED



STEP 1: SET UP 3 PCR REACTIONS:

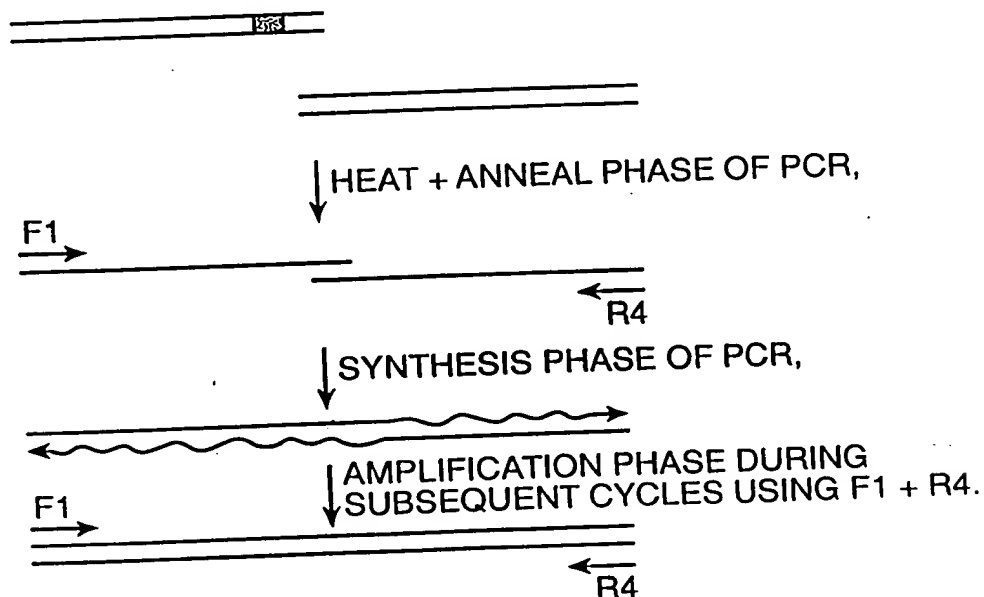
PRODUCTS:

TUBE 1:

TUBE 2:

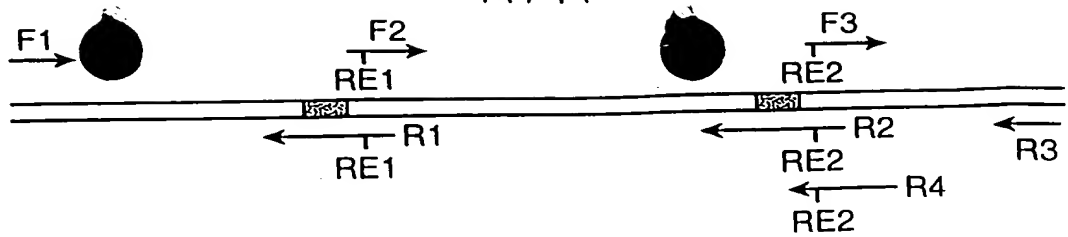
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 +
PRODUCTS TUBE 2 + F1 + R4.



STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT
FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG. 15



STEP 1: SET UP 3 PCR REACTIONS:

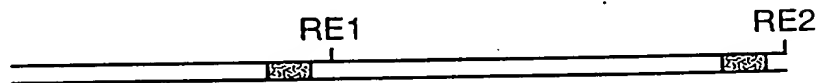
TUBE 1:

TUBE 2:

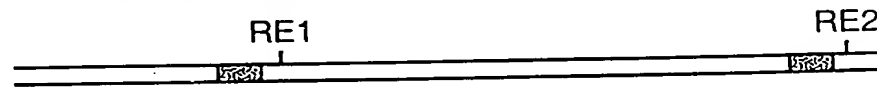
TUBE 3:

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

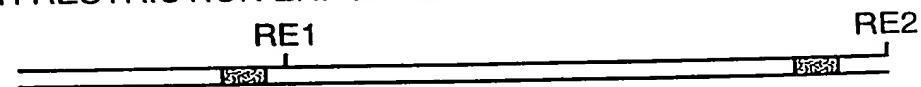
STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



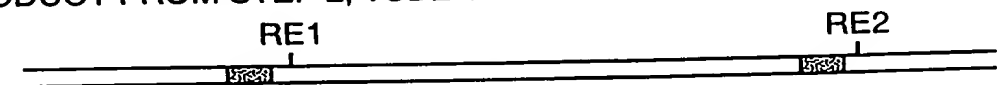
STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG. 16

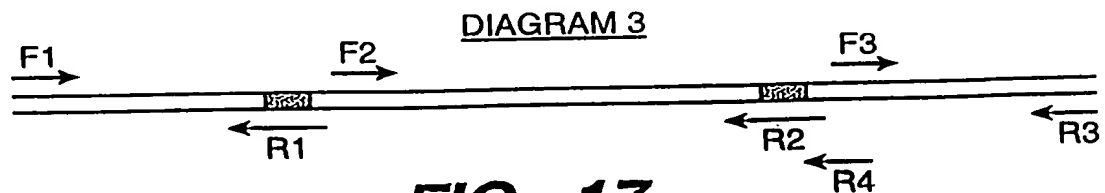


FIG. 17